Helicobacter pylori is one of the most common causes of chronic bacterial infection in humans, and is associated with many diseases of the upper gastrointestinal tract. The prevalence of infection rises with age, and in most cases colonisation probably begins during childhood. The modes of acquisition of infection and the evolution of disease associations must therefore be studied in childhood, which means that reliable non-invasive diagnostic techniques are required. The most widely used minimally invasive tool is IgG serodiagnosis, and although this does perform well among adults and older children with established infection, it may not be accurate in younger children. Stable isotope techniques may therefore prove to be more useful in early life.

In order to investigate the use of stable isotope techniques for diagnosing H pylori infection, particularly in childhood, a group of collaborators throughout Europe was established under the BIOMED initiative. The principal aims of this group were:

- To assess the performance of stable isotope tests of gastric urea hydrolysis in clinical practice as diagnostic tools for H pylori colonisation, and to evaluate protocols for their application in childhood
- To define the role of these tests as tools in clinical practice and in epidemiological studies in childhood.

The progress made in achieving these aims is summarised in this short review and in the following abstracts.

Two stable isotope substrates are available to look at urea hydrolysis—15N urea and 13C urea. Tests using these rely upon gastric hydrolysis of labelled urea by H pylori urease, and subsequent recovery of label in expired CO2 or urinary nitrogen. Although 15N urea urine tests have been described, there is little experience of their use in childhood. The group working in Leipzig have shown that this non-invasive test is acceptable among young children, and are about to undertake a study comparing the [15N]urea urine test with the 13C urea breath test (UBT) to gain an accurate picture of the sensitivity and specificity of the urine test (abstract 1).

Most workers in the field have concentrated upon the 13C UBT, which is an established tool for the diagnosis of gastric H pylori colonisation among adults. Although the same basic protocol is used in all reported studies, a wide range of possible test conditions can be employed, which may make the test more or less easy to use among children. Within this collaboration an attempt was made to define the test variables and limits of data interpretation that govern the use of the 13C UBT among children throughout Europe. An approach using a simple test meal, and comparing baseline enrichment of 13CO2 with endoscopic data in all age groups was made between several centres (abstracts 2–6, 10, and 11). This has confirmed that the 13C UBT is a reliable tool in early childhood, with specificities and sensitivities over 90%, and emphasises the test’s degree of flexibility. Test meals are not vital in childhood (abstract 2), and a range of sample collection times from 20 to 45 minutes are suitable. The test can be performed among children immediately after an anaesthetic (abstract 2) or upon ambulant children (abstracts 2, 5, and 7–11). This degree of protocol flexibility greatly enhances the usefulness of the test among young subjects.

The determination of the appropriate cut off value for discriminating between positive and negative tests is critical. This is often determined empirically by comparing breath test results with endoscopic findings. All data sets of 13C UBT results, however, contain two subpopulations: results that are positive for gastric urea hydrolysis and negative results with no evidence of urease activity. Identifying these two subpopulations, and thus establishing the most appropriate cut off, can be undertaken by analysis of the overall data set (abstracts 3 and 4), which provides a more accurate and reliable guide to the test’s performance, particularly in childhood. Comparison of the cut offs achieved by statistical or empirical means in this study (abstracts 2, 3, 5, 6, 10, and 11) shows that the choice of cut off is not age dependent, and can be fairly universally applied.

The UBT is an ideal tool for epidemiological studies (abstracts 7, 8, and 9). In childhood H pylori colonisation may be followed by spontaneous eradication (abstract 8) and there is an excess prevalence of H pylori colonisation among children with short stature and failure to thrive (abstracts 7 and 9). In clinical practice, all groups found that the UBT is an ideal method for assessing the effectiveness of eradication treatment. Two centres explored the role of the breath test in the primary diagnosis of H pylori infection as a means of screening patients before endoscopy (abstracts 10 and 11). These preliminary results suggest that a more detailed evaluation of the role of non-invasive tests of H pylori colonisation in clinical decision making before endoscopy should be undertaken.

The principal conclusions arising from this body of work on the 13C UBT are:

- A wide range of test conditions can be used and adapted to local conditions, giving great flexibility and making it relatively easy to adapt the test for use among young children.
- This degree of flexibility means that the core components of the test are simply the taking of a baseline breath sample, the administration of 13C urea in a dose sufficient to achieve

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enzyme saturation (50–100 mg in these studies, according to body size), and the collection of a second breath sample 30–45 minutes after the test dose. The comparison in 13C enrichment of expired breath between the first and second breath samples is sufficient to provide the diagnosis. The abstracts show that a range of adaptations can be made to the test provided that these core components are preserved. The selection of the test meal, timing of breath sample collection, and dose of 13C urea administered can be altered to suit local requirements, which enhances the usefulness of the test in clinical practice.

The appropriate cut off can be established from within each data set, and can be verified if necessary by empirical comparison so that conventional approaches to validating the test need not be employed. Assignment of a binary value to the test results remains consistent between different centres even with modifications to protocols, which allows true comparability of results throughout Europe in all ages.

The 13C UBT can be used to diagnose gastric H pylori colonisation in all age groups in epidemiological studies, and as a reliable means of assessing eradication after treatment.

The role of non-invasive tests in primary diagnosis of H pylori colonisation is more controversial. A full evaluation of this in terms of its influence upon clinical decision making needs to be undertaken.


(1) Helicobacter pylori infection diagnosis and treatment control in Leipzig children using the [15N]urea urine test

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Aim: To investigate the use of the [15N]urea urine test to diagnose Helicobacter pylori colonisation in children after producing a standardised protocol suitable for use among infants.

Two groups of children (aged 3–17 years) being investigated for symptoms of chronic abdominal pain were studied: (a) day patients at the paediatric polyclinic of the University of Leipzig; and (2) ambulatory patients of a local paediatrician.

On study mornings, fasting children were given a test meal of 100 g warm, slightly acidic instant pudding mixed with 3 mg [15N] urea/kg body mass. Urine samples were collected one, two, and three hours later. The two hour sample was used to analyse the [15N] content in the urinary ammonium using a non-mass spectrometric [15N] analyser (NOI-6PC, Fischer FAN, Leipzig).

Values above 0.06 for a quotient [15N] ammonium/[15N] urea in the urine samples were considered positive based on a previous study of comparisons with endoscopic diagnosis. Those children with positive results had the test repeated four weeks after treatment.

129 children with chronic abdominal pain were tested, and 49 (38%) had positive tests. Repeat testing was undertaken to check reproducibility, and a different result on the second test was only obtained in three cases. This non-invasive test has been shown to be acceptable among young children, and a study comparing the [15N]urea urine test with the 13C urea breath test in a population sample including young children is about to begin in Leipzig to gain an accurate picture of the sensitivity and specificity of the urine test, and to gain further information on the natural history of H pylori infection and its sources.

(2) 13C urea breath test for the diagnosis of Helicobacter pylori in children

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Aims: To determine if the 13C urea breath test (UBT) can be used in children by evaluating (a) its sensitivity and specificity compared with either biopsy culture or both biopsy rapid urease test and histology; (b) if a test meal or a prolonged fast are required; (c) its usefulness after treatment for Helicobacter pylori.

Eighty eight children (mean (SD) age 10.6 (4.19)) undergoing upper endoscopy were studied. Tests were performed fasting, non-fasting, and after treatment. Children were given 50 mg 13C urea (<50 kg) or 75 mg urea (>50 kg) with 50 mg of a glucose polymer solution in 7.5 ml of water. Breath samples were collected at baseline 15, 30, 45, and 60 minutes.

In 63 fasting children the UBT was 100% sensitive and 97% specific at 30 minutes using a cut off value of 3.5 δ‰ baseline enrichment. Non-fasting tests in 23 children performed between one and two hours after their usual meal were 100% sensitive and 91.6% specific. In 13 children fed directly before the UBT the sensitivity of the test was reduced to 50%. Thirty minutes was the optimal sampling time. There was a significant decrease in specificity when samples were obtained at 15 minutes, possibly due to the interference of oral urease producing organisms. The test was 100% sensitive and specific in 20 infected children after treatment for H pylori.

The UBT is a useful test for the diagnosis of H pylori infection in children. Neither a prolonged fast nor a test meal is required. The UBT is an ideal test for studies on the epidemiology of H pylori in children.

(3) 13C urea breath test is a very accurate clinical tool in the diagnosis of intragastric Helicobacter pylori infection

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Aim: To evaluate the accuracy of the UBT by means of receiver operating characteristic (ROC) curve analysis.

A total of 172 consecutive outpatients (mean (SD) age: 39.7 (14.1) years, M/F: 1.12) referred for endoscopy because of abdominal symptoms were enrolled. After an overnight fast, endoscopy was performed. Three biopsy specimens were taken from the antrum and two from the fundus of the stomach. Four specimens were stained with haematoxylin and eosin and cresyl-violet, and one antral biopsy was used for culture. A blood sample was taken from all subjects and measurement of specific antibodies for Giardia lamblia and Helicobacter pylori was performed by ELISA (Helori-test, Eurospital, Trieste, Italy). Patients were considered infected if they were positive on histology or culture, or both. The UBT was done one week after the endoscopy because of abdominal symptoms were referred for endoscopy.

Breath samples were taken before the meal and every 15 minutes for one hour after ingestion of the urea solution. The 13C enrichment was determined by isotope ratio mass spectrometry (ANCA-NT, Europa Scientific, Crewe, UK). The analytical data were expressed as percentages of 13CO2 recovery per hour of the administered dose (%DH) at 15, 30, 45, and 60 minutes. The percentage of 13CO2 cumulative values at 60 minutes (%CD) was calculated. The difference between the delta value at 30 minutes and the delta value at baseline (DOB30 or delta over baseline at 30 minutes) was also measured. To evaluate the accuracy of the UBT, a ROC curve was plotted using both the %CD values at 60 minutes and the DOB30 values.

One hundred and twenty six (73.2%) patients (M/F: 1.13) were infected. There were 125 (99.2%) patients positive on histology and one (0.8%) patient positive on culture but negative on histology. Eighty four (66.6%) patients were positive on culture and 60 (48.3%) patients were negative on histology. Eighty four (66.6%) patients were positive on culture. One hundred and thirty (90.5%) patients were positive on urease test. The table shows the sensitivity and specificity of these two cut-off points for UBT: one of 3.25‰ baseline difference between the delta values of the two populations by generating two normal distributions and using the data to determine the UBT value best separating H pylori positive and negative subjects.

The statistical study gave two optimal cut off points for UBT: one of +3.25‰, when the UBT is used for the initial diagnosis of H pylori infection, and the second of +2.75‰ when the UBT is used to confirm the efficacy of an anti-H pylori treatment. In both cases, the sensitivity and specificity of the UBT was more than 97%.

(4) Determination of the cut off point of 13C urea breath test in adults by cluster analysis

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Although the 13C urea breath test (UBT) is now widely used for non-invasive diagnosis of Helicobacter pylori infection, there is still some controversy about the adequate cut off point to use. Using receiver operating characteristic (ROC) curve analysis of UBT results v histology of gastric biopsies in 95 patients, we have recently reported the best cut off point to be +3.5‰. The goal of the present study was to confirm this cut off point by statistical analysis of a larger set of data. UBTs were performed in 696 individuals to establish the diagnosis of H pylori infection (diagnosis group), and in 1056 patients to control the efficacy of an anti- H pylori treatment (post-treatment group). A statistical technique was used to determine the UBT value best separating H pylori positive and negative distributions gaussian by logarithmic transformation of UBT values; (b) perform cluster analysis on the variable Ln (UBT value); (c) estimate the values of H pylori negative and H pylori positive distributions; (d) determine the cut off point of these two populations by generating their density of probability curves using the formula of the normal distribution. This statistical study gave two optimal cut off points for UBT: one of +3.25‰, when the UBT is used for the initial diagnosis of H pylori infection, and the second of +2.75‰ when the UBT is used to control the efficacy of an anti-H pylori treatment. In both cases, the sensitivity and specificity of the UBT was more than 97%.

(5) The 13C urea breath test to assess Helicobacter pylori eradication in childhood H pylori gastritis

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Aim: To assess accuracy of 13C urea breath test (UBT) to diagnose Helicobacter pylori eradication when performed one or two weeks (“early”) and six or 10 weeks after treatment has been stopped (“late”).

In 78 children (41 boys, 37 girls, median age 10.6 years, range 4–15) with H pylori gastritis antibiotic treatment (with omeprazole, amoxicillin, and metronidazole or clarithromycin) was given for one or two weeks. Six to 10 weeks later eradication was confirmed by gastroscopy with histology (Giemsa stain) and urease test, and 13C UBT (late) was performed. In half of the children an additional 13C UBT was performed with a test meal of 100 ml of a 10% solution of polyose (glucose polymer: Abbott,
UK and 50 mg of $^{13}$C urea, breath samples were collected before and 30 minutes later in Vacutainer tubes and shipped to Cambridge (UK) and Bologna (Italy).

Endoscopy based tests showed *H pylori* eradication in 54 (69%). "Early" $^{13}$C UBT was performed at one week in 32 patients, and at two weeks in 36 (10 patients did not show up for "early" $^{13}$C UBT) and the test was repeated at six weeks in 56 and at 10 weeks in 16 (in six patients results were not available either due to test tubes broken during shipping or the breath collection was insufficient). Of 68 "early" $^{13}$C UBTs 52 were negative (Excess $\delta^{13}$CO$_2$ excretion < 5 %) with five false negative (10%) and 16 were positive with one false positive (6%). Of 72 late $^{13}$C UBTs, 52 were negative with two false negative (4% both at six weeks) and 20 were positive without false positive results.

The $^{13}$C UBT is a simple and non-invasive test that can be used to assess *H pylori* eradication in children. Its specificity and sensitivity are satisfactory even one or two weeks after treatment has been stopped, but 10% false negative or 6% false positive results can occur. False negative results decrease to 4% when performed six weeks after treatment, but the best accuracy is obtained 10 weeks after treatment.

(7) *Helicobacter pylori* infection and failure to thrive among pre-school children

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Children aged < 5 years (mean age 25 months) attending for small bowel biopsy to investigate symptoms of persistent diarrhoea and growth faltering, without other abdominal symptoms, were studied. On the day of their biopsy, these children also underwent a $^{13}$C urea breath test (UBT), taking 50 mg of urea with a glucose polymer test meal, with breath samples collected at baseline and 30 minutes later. $^{13}$C enrichment in expired CO$_2$ was measured by isotope ratio mass spectrometry, (SIRA 10, VG Isotech, UK or ANCA, Europa Scientific, UK). The appropriate cut off was determined by comparison with age-matched children undergoing diagnostic endoscopy and using the same breath test protocols from other centres participating in the BIOMED collaboration. Collecting adequate breath samples from such young children on the same day as they attended hospital for an invasive procedure proved difficult. Successful breath tests, with sufficient volume of expired CO$_2$ collected to allow reliable analysis, were performed on 52 children undergoing small bowel biopsy: 50 were aged <5 years. More than 10% of the results were close to the cut off, in contrast with most other studies, reflecting the technical difficulties experienced in sample collection among such young children. Seven out of the 52 children (13.5%) had clear positive UBTs. Although population controls were not sought for this study, the prevalence of *H pylori* colonisation in normal children of this age in the UK is likely to be <1%, suggesting that there is an excess of *H pylori* colonisation among young children who fail to thrive.

(8) *Helicobacter pylori* infection in childhood: a disease with spontaneous eradications and recurrences?

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Aim: To evaluate whether *Helicobacter pylori* infection may undergo spontaneous eradication in children.

304 Italian children (age range: 4.5–18.5 years, M/F:1.0) were randomly selected from four Italian schools. Subjects taking antibiotics were excluded. Children were tested for *H pylori* by means of a $^{13}$C urea breath test (UBT). Breath samples were taken before and every 15 minutes for one hour after the oral intake of 200 ml of full cream cows' milk and 75 mg of $^{13}$C urea (Eurisotop, France). The $^{13}$C enrichment in breath was determined by isotope ratio mass spectrometry (ANCA-NI; Europa Scientific, Crewe, UK) and expressed as percentage of $^{13}$CO$_2$ cumulative dose at one hour. The UBT was considered positive if the cumulative dose was >1.15%. Using this cut off, the sensitivity and specificity of the UBT are 96% and 97.8%, respectively. Infected children were enrolled in the follow up study and regularly seen every six months. Children repeated the UBT at each visit.

Eighty five out of 304 (28%) children were *H pylori* infected. Forty eight out of 85 infected children (56%) participated at the follow up. After six months, five children were found negative at the repeat UBT: only one had been treated with a short course of erythromycin. At 12 months, 43 subjects were still positive, whereas one, who had become negative at six months, tested positive at one year.

(6) *Helicobacter pylori* and duodenal ulcer

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Aim: To compare different diagnostic techniques for diagnosing *Helicobacter pylori* colonisation within a busy department of adult gastroenterology, and to monitor patient progress after treatment.

Patients with endoscopically proved duodenal ulcer had *H pylori* infection detected by biopsy urease test, biopsy culture, histology, and $^{13}$C urea breath test (UBT). The use of several techniques before treatment allowed the correct definition of infection and the validation of these tests. Compared with biopsy culture, the sensitivity and specificity of the other tests were as table 1 shows.

After diagnosis and treatment, assessment of *H pylori* eradication was made by $^{13}$C UBT, four weeks after treatment was stopped (table 2).

Follow up endoscopy was performed at 12 and 24 months after treatment, or when clinical relapse was suspected. Additional $^{13}$C UBT was performed at three, six, 12, and 24 months.

During a follow up period of 14 to 24 months among 132 patients the ulcer relapse rate was 35.2% for those who remained positive for *H pylori* infection and 3.7% in *H pylori* negative patients. Reinfection was detected in only two patients (2.5%) with an annual rate of 1.2%.
(9) Helicobacter pylori infection is associated with short stature, low socioeconomic conditions, and household overcrowding

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Aims: To evaluate the prevalence rate of Helicobacter pylori infection in Italian children and to search for any difference in body weight and height between children with positive and negative H. pylori. A total of 216 Italian children (M/F: 105/111, aged 3–14 years) were tested for H. pylori infection by means of a 13C urea breath test (UBT; 200 ml of milk and 75 mg of 13C urea (99atm%); 13C enrichment in breath determined by isotope ratio mass spectrometry (ANCA-NT, Europa Scientific, Crewe, UK)). The height and weight of each subject were recorded and centile values were calculated. Composite indexes for socioeconomic class and household crowding were also determined.

Forty nine out of 216 (22.7%) children were infected. The prevalence rate of infection increased with age: Eight out of 49 (16.3%) H. pylori positive children v 13 out of 167 (7.8%) H. pylori negative children were below the 25th centile of height (p=0.09). This difference became significant in children aged 8.5 to 14 years; in this group (n=127), eight out of 31 (25.8%) infected subjects v eight out of 96 (8.3%) non-infected ones were below the 25th centile of height (p=0.02). Significant correlations were found between socioeconomic conditions, household overcrowding, and H. pylori status. By using stepwise logistic regression analysis, only the centile value of height (but not weight) was significantly related with H. pylori status in older children.

The overall prevalence rate of H. pylori infection in Italian children aged 3–14 years is 23% and increases with age. A significant difference in height was found between H. pylori positive and H. pylori negative children aged 8.5–14 years. The H. pylori infection is associated with low socioeconomic condition and household overcrowding. All these findings are consistent with the hypothesis that childhood acquired H. pylori infection may be one of the environmental factors capable of affecting growth.

(10) Urea breath test in the initial assessment of patients with upper gastrointestinal symptoms

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Aim: To relate the 13C urea breath test (UBT) results to endoscopic and histological findings in patients with upper gastrointestinal symptoms, and to evaluate the role of the UBT in clinical decision making.

A total of 172 consecutive outpatients (mean (SD) age 39.7 (14.1) years, M/F 1.12) referred for dyspepsia, and never previously endoscoped, were studied. Symptoms were scored using a questionnaire. All patients underwent endoscopy with biopsies (histology with haematoxylin and eosin and cresyl violet, Sydney score) and UBT. One antral biopsy was used for culture. Patients positive on histology and/or culture were considered infected.

Results: 126 (73.2%) patients were H. pylori positive. Of these, 124 were H. pylori positive on antral biopsies. The sensitivity and specificity of the UBT were 96% and 97.8%. Table 1 gives the endoscopic findings.

Either gastroduodenal ulcers or cancers and histologically severe lesions (panmucosal gastritis; mononuclear and PMN infiltration; metaplasia and atrophic changes) were almost exclusively observed in H. pylori positive patients. In H. pylori negative patients, the only endoscopic abnormalities seen were non-specific gastritis or duodenitis with super¬ficial inflammation on histology (table 2).

A significant association was found between H. pylori positivity and belching, and between H. pylori negativity and mucus in the stool. Conclusions: The syndrome of dyspepsia is generally associated with gastritis, peptic ulcer, or gastric cancer. Because the risk of gastric cancer is estimated as extremely low in people aged <45 years, we suggest that in younger dyspeptic patients, the UBT could be used to screen subjects before endoscopy. Endoscopy should be reserved for H. pylori negative patients, or patients who remain symptomatic after H. pylori eradication.

(11) Use of the 13C urea breath test for the diagnosis of Helicobacter pylori infection in childhood

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A total of 143 children, age range 1–15 years undergoing upper gastrointestinal endoscopy to investigate symptoms relating to upper gastrointestinal disease were studied. Biopsies were taken from the gastric antrum, and the presence of Helicobacter pylori determined by rapid urease test, histology (including Giemsa stain), and culture of bacteria from biopsies in some cases. 13C urea breath tests (UBTs) were performed on the children on the day of the endoscopy, using either 50 mg of 13C abundant urea (99atm%) with a glucose polymer test meal, or 75 mg of 13C urea with 200 ml of milk. Breath samples were collected at baseline and at 30 minutes after taking the urea. Results above a cut off of 4.5 % relative to an
international $^{13}$C standard PDB above baseline at 30 minutes were considered positive, although adjusting this cut off to any value between 4 and 5.5 did not significantly affect the results. Table 1 shows the number of children with positive identification of *H pylori* compared with UBT results.

Only one *H pylori* positive child was aged <5 years. The sensitivity of the UBT compared with biopsy identification was 79% and the specificity 96%. Eight of the 11 false negative tests occurred in the first few months of the study, and could reflect problems encountered in establishing the test. It is also possible that changes made in the test protocol resulted in the lower rate of false negative tests seen in the latter part of the study. All *H pylori* positive children underwent eradication treatment. If the UBT had been used as a screening test among these children, with eradication being offered to positive children and endoscopy to negative children, three children would have been treated unnecessarily, all infected children would have been treated, and 44/143 (31%) would not have required endoscopy. One of these 44 also had coeliac disease. Among the 99 children with negative UBTs, coeliac disease was diagnosed at endoscopy in 11, oesophagitis in 25, and superficial gastritis in 22.